

B2  
cont

fragment of PTPZ containing at least a region within the amino acid sequence 1420-1780 of said PTPZ protein.

### REMARKS

In view of the above amendments and the following remarks, reconsideration of the outstanding office action is respectfully requested.

Claims 1-4 and 44-45 are pending. Claims 1-4 and 44-45 were objected to for containing non-elected subject matter. Claims 1 and 3 were amended and claims 4 and 44-45 were cancelled to remove non-elected subject matter. Support for the amendments is found in the originally filed application. The amendments do not constitute addition of new matter. Pursuant to 37 CFR §1.121, attached as an appendix is a version of the amendments with markings to show changes that have been made. In light of the amendment to claim 1 and the cancellation of claims 44 and 45, the Office Action's objection to claims 1-4 and 44-45 for containing non-elected subject matter should be withdrawn.

#### I. Utility Rejection Under 35 U.S.C. §101

The PTO rejected claims 1-4 and 44-45 under 35 U.S.C. §101 for allegedly having neither a specific and substantial utility nor a well-established utility. Specifically the Office Action alleges that the present application fails to disclose any particular function or specific biological significance for the protein complex comprising IRAP and PTPZ. Applicants respectfully traverse.

##### A. Particular and Specific Biological Significance of IRAP-PTPZ Interaction

Numerous studies have indicated that there is a link between insulin resistant diabetes (Type II) and Alzheimer's disease, but the biochemical nature of this connection is heretofore not well understood. Gasparini *et al.*, *Trends Pharmacol Sci*, 23:288-293 (2002); Hoyer, *J Neural Transm*, 109:341-360 (2002); Hoyer, *J Neural Transm*, 109:991-1002 (2002). The discovery of the protein-protein interaction between IRAP and PTPZ provides such a molecular link. Specifically, IRAP is implicated in diabetes through its association with the insulin-regulated glucose transport protein Glut4. Another insulin-regulated protein—secreted APP (“sAPP”)—

binds the signal transduction phosphatase PTPZ, thereby stimulating neuroprotective effects, which are notably absent in Alzheimer's disease patients. Therefore, the discovery that IRAP and PTPZ interact with each other, points to the likelihood that the co-occurrence of non-insulin dependent diabetes and Alzheimer's disease may be treated by modulating the interaction between IRAP and PTPZ (See Exhibit A, attached).

Central to the question of how to treat non-insulin dependant diabetes mellitus is how glucose uptake is regulated in the cell. Toward this end, there has been much interest in analyzing the Glut4 protein and its glucose transport activities. In addition to being present in the relevant and relatively restrictive sites for glucose disposal (muscle and adipose), Glut4 is also preferentially expressed in various regions of the brain although its precise function in CNS tissue is not well understood. Unlike other related glucose transporters, Glut4 is regulated by insulin. Thus, insulin responsiveness of the brain is likely to be attributed at least in part to Glut4. IRAP shows similar tissue distribution to Glut4 and is expressed in the CNS. IRAP completely co-localizes with the Glut4 transporter in specified endocytic vesicles and at the plasma membrane. Keller *et al.*, *J Biol Chem.* 270:23612-8 (1995); Malide *et al.*, *FEBS Lett.* 409:461-8 (1997). Moreover, expression of the N-terminal fragment of IRAP has been shown to result in the translocation of Glut4 to the plasma membrane. Waters *et al.*, *J Biol Chem.* 272:23323-7 (1997).

PTPZ is implicated in Alzheimer's disease through its interaction with the sAPP. U.S. Publication Application No. 20020069424. Insulin stimulates  $\alpha$ -secretion of APP, which results in reduced generation of the A $\beta$  peptide, the toxic form of APP. sAPP exhibits neuroprotective effects, which are likely mediated by a receptor that activates a signal transduction cascade. PTPZ is a likely receptor for sAPP. Krueger *et al.*, *EMBO J* 9:3241-3252 (1990); Krueger and Saito, *Proc Natl Acad Sci* 89:7417-7421 (1992); Levy *et al.*, *J Biol Chem* 268:10573-10581 (1993); Snyder *et al.*, *Brain Res Mol Brain Res* 40:79-96 (1996). Further evidence that PTPZ is essential to the neuroprotective effects of sAPP is in the fact that PTPZ is expressed specifically in the central nervous system and its level of expression is increased after brain injury. Levy *et al.*, *J Biol Chem* 268:10573-10581 (1993); Snyder *et al.*, *Brain Res Mol Brain Res* 40:79-96 (1996). Because APP metabolism and function, as well as phosphorylation reactions, are deeply disrupted in the brain of Alzheimer's patients, and because sAPP activities at the cellular level

(neurotrophic, neuroprotective) are reflected by memory enhancement at the behavioral level, drugs that alter PTPZ activity will have a tremendous potential for the treatment of neurodegenerative disease, particularly Alzheimer's disease.

Using the yeast two-hybrid system, applicants have detected the interaction of IRAP with PTPZ, one protein linked directly to the biology of diabetes and another linked to Alzheimer's disease. Yet, both proteins belong to cellular pathways regulated by insulin and insulin signaling. As is well known in the art, numerous studies reveal the effects of insulin on the molecular and cellular mechanisms that underlie both the pathology of diabetes and Alzheimer's disease. Thus, the discovery of a protein complex comprising IRAP and PTPZ provides a molecular bridge connecting the functions of the insulin-responsive glucose transporter Glut4 with the neurotrophic sAPP protein. Given that both PTPZ and IRAP are localized to the plasma membrane, it is likely that PTPZ modulates the activity of IRAP, and thereby plays a role in glucose uptake in response to sAPP binding. IRAP might also influence the subcellular localization or function of PTPZ in response to insulin. Therefore, the interaction between a protein linked to diabetes with a second protein linked to Alzheimer's disease provides a unique and novel insight into the important connection between diabetes and Alzheimer's disease, and ultimately sheds light on a number of novel methods of therapeutic interventions for these diseases.

As stated in the specification of the present application, and as is well known in the art, "[m]any processes in biology, including transcription, translation and metabolic or signal transduction pathways, are mediated by non-covalently associated protein complexes" (page 1, lines 25-26). Consequently, the discovery of a novel protein-protein interaction provides not only valuable insight into complex biological processes, but also serves as an important target for the development of therapeutics that modulate the protein-protein interaction, particularly when the individual proteins are implicated in important cellular or disease pathways. Applicants disclosed that the protein-protein interaction between IRAP and PTPZ are physiologically relevant, and that the modulation of the interaction is useful in the treatment of specific diseases, including diabetes and Alzheimer's disease. Accordingly, applicants respectfully disagree with the PTO's assertion that the present application fails to disclose any particular function or specific biological significance for the protein complex comprising IRAP and PTPZ.

## B. Burden of Proof

Applicants respectfully remind the PTO that an applicant "does not have to provide evidence sufficient to establish that an asserted utility is true 'beyond a reasonable doubt.'" M.P.E.P. §2107.02 (VII). All that is required of applicants is evidence that it is more likely than not that the asserted utility is specific or substantial to a person of ordinary skill in the art. *Id.* Accordingly, applicants submit that the protein-protein interaction discovered by the methods of the yeast two-hybrid assay provide enough evidence that the IRAP-PTPZ interaction provides a specific and substantial utility. Particularly, applicants assert that the IRAP-PTPZ protein complex of the present application provides a molecular link between Alzheimer's disease and diabetes, as has been discussed above.

The yeast two-hybrid assay employed in the discovery of the IRAP-PTPZ protein complex is designed to identify genes encoding proteins that physically associate with a given protein *in vivo*. Bartel and Fields, eds., *The Yeast Two-Hybrid System*, Oxford University Press, New York, NY, 1997. Because the assay identifies interactions occurring within a eukaryotic yeast cell, the proteins are expected to be under the same physiological conditions as in a mammalian cell. Moreover, because the host cell is yeast, "endogenous yeast proteins are less likely to bind a mammalian target protein to prevent its interaction with a protein encoded by a library." Fields *et al.*, *Trends in Genetics* 10:286-292 (1994). Therefore, the yeast two-hybrid system is actually a better environment in which to perform the protein-protein interaction than the original mammalian cell. On this basis, a skilled artisan reasonably expects that a protein interaction identified in the yeast two-hybrid system is a protein interaction that occurs *in vivo* in a mammalian cell, and therefore possesses specific and substantial utility.

Notably, the yeast two-hybrid assay is a method of choice for detecting physiologically relevant protein-protein interactions (*See Exhibit B*). Although there are other methods for detecting protein-protein interactions, "the development of the yeast two-hybrid system appears to have had the greatest impact on interaction cloning methodology." Bartel and Fields, eds., *The Yeast Two-Hybrid System*, Oxford University Press, New York, NY, 1997. Specifically, the yeast two-hybrid system was instrumental in elucidating cell cycle control pathways in mammalian cells by confirming relevant protein interactions isolated simultaneously by multiple

other approaches, and in other cases being the only method of discovering key protein interactions involved in cell cycle control. *Id.*

The Office Action has not met its burden of establishing a *prima facie* showing that the asserted utility is not specific or substantial. The PTO's *prima facie* case must: (1) clearly explain the reasoning why the utility is not specific and substantial; and (2) provide an explanation that clearly sets forth the reasoning used in concluding that the asserted utility for the claimed invention is not specific and substantial. M.P.E.P. §2107.02 (IV). ("Where the asserted utility is not specific or substantial, a *prima facie* showing must establish that it is more likely than not that a person of ordinary skill in the art would not consider that any utility asserted by the applicant would be specific and substantial." M.P.E.P. §2107.02 (VII).) The Office Action states merely that "the prior art of record demonstrates that the biological function of the protein family to which the disclosed protein is said to be a member is so diverse, that one could not predict which biological activity is possessed by the disclosed protein complex based on the state of the prior art with respect to the already complex physiological function of each of the two proteins." Office Action, pg. 4. However, the fact that the proteins of the claimed complex are involved in complex physiochemical interactions and have been shown to interact with other proteins says nothing with respect to the credibility of the claimed protein complex's specific and substantial utility. The discovery of one protein-protein interaction does not discount the occurrence of other protein-protein interactions involving the same proteins. In fact, proteins are known to form complex interactions with a variety of proteins. Because the present application discloses a specific and credible interaction between two proteins, the interaction of which provides a molecular link between two important diseases, one who is skilled in the art would likely conclude that the disclosed utilities are specific and substantial.

Accordingly, applicants respectfully request that the PTO's rejection of claims 1-4 and 44-45 for lack of utility under 35 U.S.C. §101 be withdrawn.

## **II. The Enablement Rejection Under 35 U.S.C. §112, First Paragraph**

Claims 1-4 and 44-45 are also rejected under 35 U.S.C. §112, first paragraph, for allegedly containing subject matter not described in the specification in such a way as to enable one skilled in the art to which it pertains. Applicants respectfully traverse.

As discussed above, the interaction between IRAP and PTPZ provides a molecular link between diabetes and Alzheimer's disease. The interaction constitutes an ideal drug target for therapeutic intervention for the treatment of diabetes and/or Alzheimer's disease. That is, modulation of the IRAP-PTPZ interaction would likely lead to the treatment of the diseases. Accordingly, isolated protein complexes formed by the IRAP-PTPZ interaction are useful in selecting modulators of the interaction which can be developed into drugs for the effective treatment of diabetes and/or Alzheimer's disease.

Methods for identifying modulators of protein-protein interactions are well known in the art. For example, Vidal *et al.*, *Proc. Natl. Acad. Sci. USA*, 93:10315-10320 (1996) teaches the so called "reverse two-hybrid system" for screening dissociators of protein-protein interactions. See Exhibit C. Young *et al.*, *Nature*, 16:946-950 (1998) is an example in which a modulator of protein-protein interaction was identified by the reverse yeast two-hybrid screen. See Exhibit D and E. Further, Degterev *et al.*, *Nature, Cell Biol.*, 3:173-182 (2001) discloses the identification of small-molecule inhibitors of protein-protein interactions using a high-throughput screen based on fluorescence polarization (FP). See Exhibit F. In addition, the Specification of the instant application also specifically provides methods for selecting modulators of protein-protein interactions. See Specification, pages 34-35, and particularly page 36. Therefore, methods for utilizing isolated protein complexes comprising IRAP interacting with PTPZ in screening assays for selecting modulators of the interaction would be apparent to an ordinarily skilled person in the art, in view of the disclosure in the Specification.

The Office Action further rejects Claim 3 under 35 U.S.C. §112, first paragraph, for lack of enablement on how to make and use any fragment of IRAP and/or PTPZ in the claimed protein complex. Claim 3 has been amended to recite that the protein fragments contain amino acid sequences with the specified regions of the full-length proteins. To the extent the §112 rejection would be similarly applied to the amended claim, Applicants respectfully traverse for the following reason:

As disclosed in the Specification, Example 69 (see page 57), the amino acid sequence encoded by nucleotides 62-880 (i.e., amino acid residues 1-273) of IRAP cDNA is sufficient to enable IRAP protein to interact with PTPZ. Similarly, the amino acid residues 1420-1780 of PTPZ are sufficient for PTPZ to bind IRAP. Accordingly, any IRAP protein fragments including the

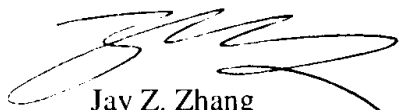
amino acid residues 1-273 would be capable of interacting with PTPZ. Likewise, any PTPZ protein fragments comprising the amino acid residues 1420-1780 would be able to bind to IRAP.

In addition, IRAP fragments capable of interacting with PTPZ can also be identified by the combination of molecular engineering of an IRAP-encoding nucleic acid and a method for testing protein-protein interaction. For example, the coordinates in Example 69 can be used as starting points and various IRAP fragments falling within the coordinates can be generated by deletions from either or both ends of the coordinates. The resulting fragments can be tested for their ability to interact with PTPZ using any methods known in the art for detecting protein-protein interactions (e.g., yeast two-hybrid method). Alternatively, various IRAP fragments can be made by chemical synthesis. The IRAP fragments can then be tested for its ability to interact with PTPZ using any method known in the art for detecting protein-protein interactions. Examples of such methods include protein affinity chromatography, affinity blotting, *in vitro* binding assays, yeast two-hybrid assays, and the like. Likewise, PTPZ fragments capable of interacting with IRAP can also be identified in a similar manner. It is respectfully submitted that the recombinant DNA methods and methods for testing protein-protein interactions are routine in the art, and by no means requires "undue experimentation."

Therefore, Applicants respectfully submit that ordinarily skilled persons in the art have been sufficiently enabled to use fragments of PTPZ and IRAP for forming the claimed protein complexes. The enablement rejection under 35 U.S.C. §112, first paragraph, should be withdrawn.

In view of the foregoing it is respectfully submitted that this application is now in condition for allowance, which action is respectfully requested. The Examiner is invited to telephone the undersigned to expedite allowance of this application.

Respectfully submitted,



Jay Z. Zhang  
Registration No. 44,003)

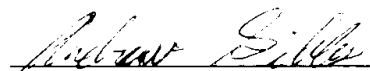
Intellectual Property Department  
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Andrew Gibbs

12/30/02

Date



## APPENDIX

In reference to the amendments made herein to the application, additions appear as underlined text while deletions appear as double strikethrough text, as indicated below:

### In the Claims:

1. (Amended) An isolated protein complex comprising two proteins, ~~the protein complex selected from the group consisting of~~ wherein one of said proteins is Insulin-Regulated Membrane-Spanning Aminopeptidase (IRAP) and the second protein is Protein Tyrosine Phosphatase Zeta (PTPZ).

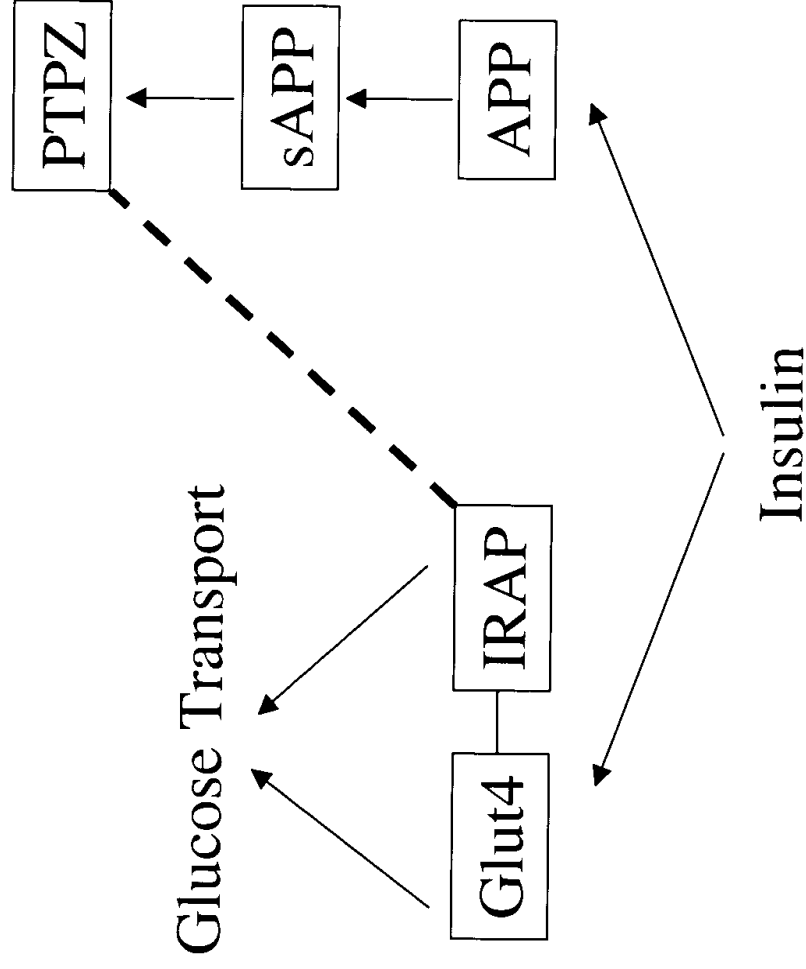
- ~~(a) a complex set forth in Table 1;~~
- ~~(b) a complex set forth in Table 2;~~
- ~~(c) a complex set forth in Table 3;~~
- ~~(d) a complex set forth in Table 4;~~
- ~~(e) a complex set forth in Table 5;~~
- ~~(f) a complex set forth in Table 6;~~
- ~~(g) a complex set forth in Table 7;~~
- ~~(h) a complex set forth in Table 8;~~
- ~~(i) a complex set forth in Table 9;~~
- ~~(j) a complex set forth in Table 10;~~
- ~~(k) a complex set forth in Table 11;~~
- ~~(l) a complex set forth in Table 12;~~
- ~~(m) a complex set forth in Table 13;~~
- ~~(n) a complex set forth in Table 14;~~
- ~~(o) a complex set forth in Table 15;~~
- ~~(p) a complex set forth in Table 16;~~
- ~~(q) a complex set forth in Table 17;~~
- ~~(r) a complex set forth in Table 18;~~
- ~~(s) a complex set forth in Table 19;~~
- ~~(t) a complex set forth in Table 20;~~
- ~~(u) a complex set forth in Table 21;~~

~~(v) a complex set forth in Table 22;~~  
~~(w) a complex set forth in Table 23;~~  
~~(x) a complex set forth in Table 24;~~  
~~(y) a complex set forth in Table 25;~~  
~~(z) a complex set forth in Table 26;~~  
~~(aa) a complex set forth in Table 27;~~  
~~(ab) a complex set forth in Table 28;~~  
~~(ac) a complex set forth in Table 29;~~  
~~(ad) a complex set forth in Table 30;~~  
~~(ae) a complex set forth in Table 31;~~  
~~(af) a complex set forth in Table 32;~~  
~~(ag) a complex set forth in Table 33;~~  
~~(ah) a complex set forth in Table 34;~~  
~~(ai) a complex set forth in Table 35;~~  
~~(aj) a complex set forth in Table 36;~~  
~~(ak) a complex set forth in Table 37;~~  
~~(al) a complex set forth in Table 38;~~  
~~(am) a complex set forth in Table 39;~~  
~~(an) a complex set forth in Table 40;~~  
~~(ao) a complex set forth in Table 41;~~  
~~(ap) a complex set forth in Table 42;~~  
~~(aq) a complex set forth in Table 43;~~  
~~(ar) a complex set forth in Table 44;~~  
~~(as) a complex set forth in Table 45;~~  
~~(at) a complex set forth in Table 46;~~  
~~(au) a complex set forth in Table 47;~~  
~~(av) a complex set forth in Table 48;~~  
~~(aw) a complex set forth in Table 49;~~  
~~(ax) a complex set forth in Table 50;~~  
~~(ay) a complex set forth in Table 51;~~

~~(az) a complex set forth in Table 52;~~  
~~(ba) a complex set forth in Table 53;~~  
~~(bb) a complex set forth in Table 54;~~  
~~(be) a complex set forth in Table 55;~~  
~~(bd) a complex set forth in Table 56;~~  
~~(be) a complex set forth in Table 57;~~  
~~(bf) a complex set forth in Table 58;~~  
~~(bg) a complex set forth in Table 59;~~  
~~(bh) a complex set forth in Table 60;~~  
~~(bi) a complex set forth in Table 61;~~  
~~(bj) a complex set forth in Table 62;~~  
~~(bk) a complex set forth in Table 63;~~  
~~(bl) a complex set forth in Table 64;~~  
~~(bm) a complex set forth in Table 65;~~  
~~(bn) a complex set forth in Table 66;~~  
~~(bo) a complex set forth in Table 67;~~  
~~(bp) a complex set forth in Table 68;~~  
~~(bq) a complex set forth in Table 69;~~  
~~(br) a complex set forth in Table 70;~~  
~~(bs) a complex set forth in Table 71;~~  
~~(bt) a complex set forth in Table 72;~~  
~~(bu) a complex set forth in Table 73.~~

3. (Amended) The protein complex of claim 1, wherein said protein complex comprises a ~~fragment of one protein and a complete protein of another protein~~ first protein which is a complete IRAP protein or a fragment of IRAP protein containing at least a region within the amino acid sequence encoded by the nucleotide sequence 62-880 of said IRAP protein; and a second protein which is a complete PTPZ protein or a fragment of PTPZ containing at least a region within the amino acid sequence 1420-1780 of said PTPZ protein.

# Exhibit A



## Searching for Interacting Proteins with the Two-Hybrid System I

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Chang Bai  
Stephen J. Elledge

Protein-protein interactions have attracted much attention because they form the basis of a wide variety of biochemical reactions. The identification of proteins that interact with a known protein is an essential aspect of the elucidation of the regulation and function of that protein. This interest has stimulated the development of a number of biochemical and genetic approaches to identify and clone genes encoding interacting proteins including coimmunoprecipitation, copurification, cross-linking, and direct expression library screening using proteins as probes. However, the development of the yeast two-hybrid system appears to have had the greatest impact on interaction cloning methodology.

The yeast two-hybrid system was devised to identify genes encoding proteins that physically associate with a given protein *in vivo*. This is a versatile and powerful method that is applicable to most, if not all, proteins once their genes have been isolated. In contrast to biochemical methods detecting protein-protein interaction, this system is based on a yeast genetic assay in which the interaction of two proteins is measured by the reconstitution of a functional transcription activator in yeast (Fields and Song 1989; Chien et al. 1991). This method not only allows identification of proteins that interact, but also can be used to define and/or test the domains/residues necessary for the interaction of two proteins (Li and Fields 1993). Since its development, a large number of genes from a variety of studies have been

## Two-hybrid Screening and the Cell Cycle

Gregory J. Hannon

### Cell Cycle Control in Mammalian Cells

Unlike many other reviews in this volume, this chapter does not present technical advice on the execution of the two-hybrid screen or its variants. Instead, it focuses on the impact that the two-hybrid approach has had on a single area of research. The mechanics of cell cycle control have been intensively studied, both because of the ubiquitous importance of this process in biology and because of the probable connection to human disease. This was one of the first fields of inquiry in which the two-hybrid screen found widespread use and had broad impact.

Progression through the cell cycle is controlled by a family of evolutionarily conserved enzymes known as the cyclin-dependent kinases or CDKs (see Sherr 1994; Hunter and Pines 1994). These proteins are not active on their own but instead require association with a positive regulatory subunit called a cyclin (see Draetta 1990). Cyclin-dependent kinases were first discovered by genetic methods in the fission yeast, *Schizosaccharomyces pombe*, and in the budding yeast, *Saccharomyces cerevisiae* (see Draetta 1990). In these organisms, a single cyclin-dependent kinase, *cdc2* in *S. pombe* or *Cdc28p* in *S. cerevisiae*, controls progress through the entire division cycle. In mammalian cells, the situation is more complex. The CDK family in human cells consists of at least seven members: CDC2

Table 11-1 Cell cycle regulators cloned by two-hybrid screening.

Function	Gene	Target	Reference
<i>CDK4/CDK6 inhibitors</i>			
	p16	CDK4	Serrano et al. 1993
	p15	CDK6	Guan et al. 1994
	p18	CDK4	Hirai et al. 1995
		CDK6	Guan et al. 1994
	p19	CDK4	Hirai et al. 1995
		Nur77	Chen et al. 1995
<i>Rb family member</i>			
	p130	CDK2	Hannon et al. 1993
<i>Rb phosphatase</i>			
	PP1- $\alpha$ 2	Rb	Durfee et al. 1993
<i>Rb-binding transcription factors</i>			
	E2F-4	p130	Sardet et al. 1995
			Beijersbergen et al. 1995
	E2F-5	p130	Hijmans et al. 1995
			Sardet et al. 1995
<i>General CDK inhibitors</i>			
	p21	CDK2	Harper et al. 1993
	p27	cyclin D1	Toyoshima and Hunter, 1994
<i>CAK cyclin</i>			
	cyclin H	CAK	Makela et al. 1994
<i>CDK Thr 161 phosphatase</i>			
	KAP	CDK2	
		CDC2	Hannon et al. 1994
	CDI1	CDK2	Gyuris et al. 1993

### Conclusions

In this review, I have detailed three pathways in which the two-hybrid approach has contributed to our understanding of the mechanisms that control the cell division cycle (see Table 11-1). In some cases, the relevant proteins were isolated almost simultaneously by multiple approaches (for example, p21, p130). In others, the two-hybrid screen provided our only access to key regulators (for example, p16, KAP/CDI1). In the past, the identification of new cell cycle regulators required the forethought, patience, and precision